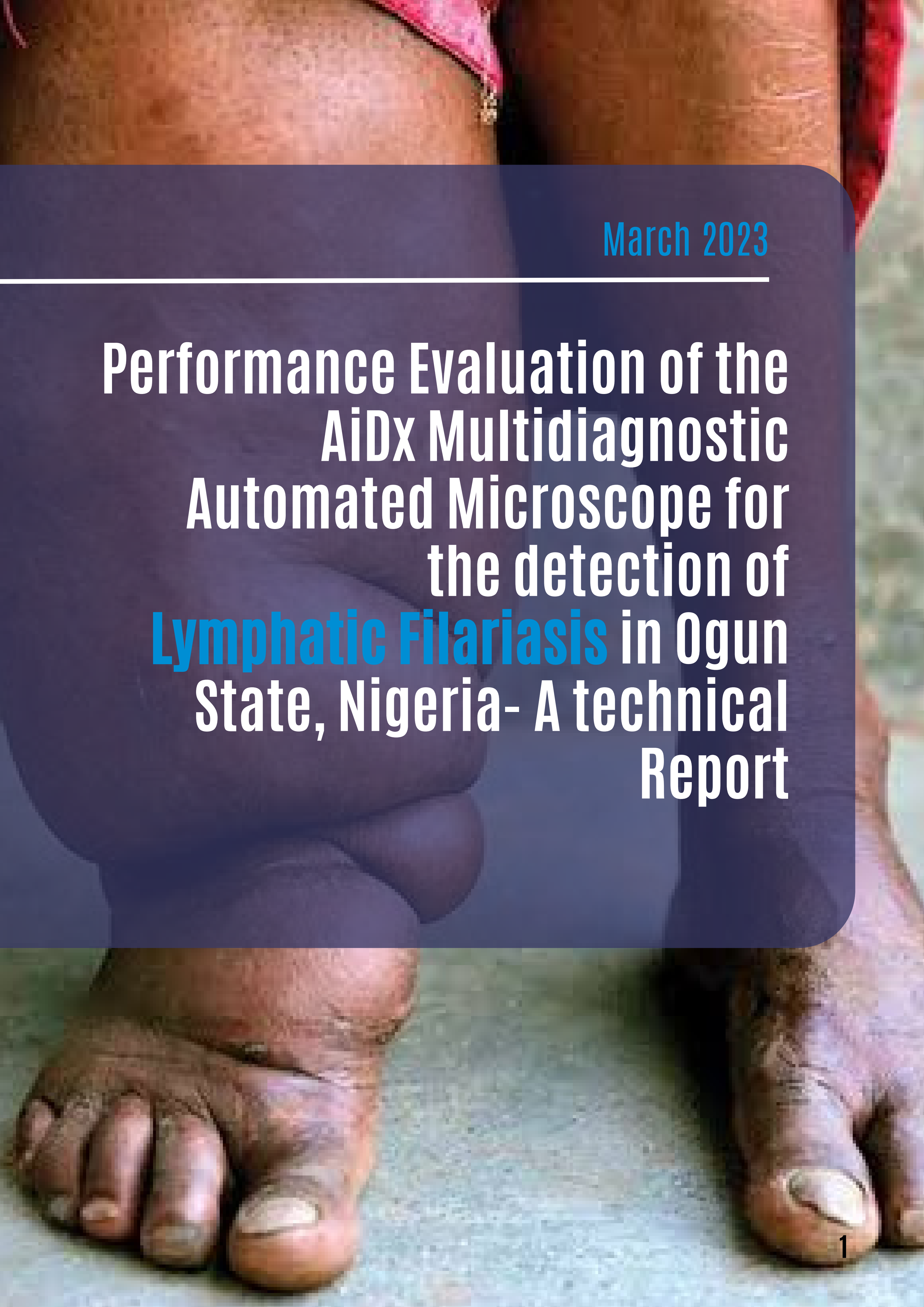
Performance Evaluation of the AiDx Multi-Diagnostic Automated Microscope For the Detection of Lymphatic Filariasis in Ogun State, Nigeria

A Technical Report

Makau-Barasa, Louise ; Aderogba, Moses O. ; Bell, David; Popoola, Samuel ; Agbana, T.E.; Diehl, J.C.; Vdovin, Gleb



March 2023

Performance Evaluation of the AiDx Multidiagnostic Automated Microscope for the detection of Lymphatic Filariasis in Ogun State, Nigeria- A technical Report

Contributors

Louise Makau-Barasa [1]
Moses Aderogba [2]
David Bell [2]
Samuel Popoola [6]
Temitope Agbana* [6,7]

Research Team

Louise Makau-Barasa [1]
Liya Assefa [1]
Moses Aderogba [1]
David Bell [2]
Jacob Solomon [3]
Nse Akpan [3]
Soneye Islamiat [4]
Dung D Pam [5]
Jospeh Kumbur [6]
Julia A-Enegela [6]
James G.Damen [7]
Samuel Popoola [8]
Jan-Carel Diehl [9]
Gleb Vdovine [8,9]
Temitope Agbana [6,7]

Correspondence:

Temitope Agbana
AiDx Medical BV
Weteringweg 2k,
Pijnacker, The Netherlands
Email: t.e.agbana@tudelft.nl

Acknowledgements:

Ivan Eikelenboom [8]
Satyajith Jujjavarapu [8]
Alexandra Bunda [8]

[1] The Ending Neglected Diseases (END) Fund, New York, United States
[2] Independent consultant,Texas, United States
[3] NTD Division, Federal Ministry of Health, Abuja, Nigeria
[4] NTD Division, Ogun state ministry of health, Ogun, Nigeria
[5] Applied Entomology and Parasitology Unit, University of Jos, Jos, Nigeria
[6] CBM International, Cambridge, United Kingdom
[7] Medical Lab Department, University of Jos, Nigeria
[8] AiDx Medical Bv, Pijnacker, The Netherlands
[9] Delft University of Technology, Delft, The Netherlands

ABSTRACT

To achieve the WHO's goal of sustained elimination of Lymphatic Filariasis disease, there is the need for accurate diagnostic device especially in areas of low-intensity infections. This project, which is a collaboration of the Nigerian Federal Ministry of Health (FMoH), AiDx and funded by THE END FUND is exploring the performance capacity of the novel AiDx_NTDx Assist automated microscope in the detection of microfilaria – Wuchereria bancrofti in prepared blood samples (glass slides) using standard microscopy as a reference. The prevalence of circulating Wuchereria bancrofti microfilaria in blood was assessed using automated Image based microscopy analysis for the detection of microfilariae in prepared blood smears.

1250 people in four LGAs of Ogun state, Nigeria participated in this study. All prepared blood samples analyzed by both expert manual microscopy and the AiDx NTDx Assist results showed that none of the 1250 participants samples analyzed had any presence of W. bancrofti microfilariae in their blood. Since no positive samples was detected by the reference test and the AiDx NTDx Assist, it was impossible to estimate the sensitivity of the device. However, based on the negative results obtained, the AiDx NTDx Assist showed a specificity of 100%, an accuracy of 100% and a Negative Predictive Value of 100%.

Despite the baseline report obtained from the National data base of the ministry of health, indicating the prevalence of 10%, 8.2%, 4.2% and 4% in the four local government areas where samples were collected, we were not able to find a participant with detectable microfilaria. Evaluation of the AiDx NTDx Assist device shows direct correlation with the expert manual microscopy. Although samples were taken in remote/rural areas of some of the LGA, e.g., Adodo Ota, result obtained however suggest a deviation from baseline and reality. This may be due to previous MDA undertaken in 2018 as reported by the state NTD officers. Further, thorough reassessment is therefore recommended.

INTRODUCTION

Lymphatic filariasis (LF) is a disease caused by Nematode worms that live in the lymphatic vessels of humans. It is a significant health problem in many developing countries. In sub-Saharan Africa, 512 million people are at risk of being infected with LF, while 28 million are known to be infected [1]. Approximately 1.4 billion people are known to live in endemic areas, with one quarter of the population potentially infected [2,3]. It is also the second leading cause of permanent disability significantly undermining the socio-economic welfare of affected people and communities [4]. There are three species of filarial parasites which infect humans—*Wuchereria bancrofti, Brugia malayi* and *Brugia timori*. About 23 species of mosquitoes including *Anopheles, culex, Mansonia, Aedes* and *Armager* are known to transmit filariasis [4].

Although mortality from LF is low, the disease is the fourth leading cause of Disability Adjusted Life Years (DALYs) (1,2). World Health Organisation (WHO) ranks LF as the second common cause of long-term disability after mental illness (3,4). Also, Persons with LF particularly women and children become more susceptible to secondary infection with HIV/AIDS, tuberculosis, and malaria (1).

Nigeria is ranked the third most endemic country globally, therefore LF is a serious public health concern in Nigeria. Annual mass treatment with single-dose diethylcarbamazine (DEC) or ivermectin (IVM) in combination with albendazole (ALB) for 4–6 years is the principal tool of the elimination strategy [5].

LF is known to cause a wide range of clinical and sub-clinical symptoms. It is estimated that two-thirds of infected individuals present no obvious proof of the disease. Bancroftian and brugian filariasis are typically characterized by different clinical manifestations varying from asymptomatic microfilaremia (where part of the population in an endemic area shows no recognizable clinical manifestation of filariasis) to chronic manifestations (clinical pathological consequences of chronic bancroftian filariasis) are clearly visible in infected patient.

Diagnostic method used to monitor and measure programs and interventions to mitigate the prevalence of LF include methods that test for the presence of circulating filaria antigen (CFA) for *W. bancrofti* and antifilarial antibodies (BmR1) for *Brugia Spp*. The Alere Filariasis Test Strip (FTS; Abobott, United states) measures CFA and Brugia Rapid Test (BRT; Reszon Diagnostics, Malaysia) measures antifilarial antibodies. Measuring CFA alone may not be an adequate way to monitor LF elimination programs. CFA can take 12 months or more to appear after incident infection and may persist several months to years after adult worms can no longer reproduce or have died [6,7,8].

Similarly, the presence of antifilarial antibodies may be indicative of prior exposure to *Brugia spp*. And may not represent an active infection [9-12]. The standard method for diagnosing active infection is the microscopy examination and identification of microfilariae in a blood smear.

This diagnostic technique is however time-consuming, laborious, expert-dependent, and error-prone manual read-out. *W. bancrofti and the Brugia* species exhibit nocturnal periodicity, therefore optimum time for blood collection is between 10:00 pm and 2:00 am.

To achieve the WHO's goal of sustained elimination, there is need for a reliable automated diagnostic device for the detection of the disease especially in areas of low-intensity infections. Artificial Intelligence based Digital Pathology can contribute to NTD programs and intervention since they still significantly rely on visual and microscopic examinations of specimens. This digitalized workflow can potentially reduce operational costs via increased throughput, automated data registration, analysis, reporting, storage, and mapping.

The aim of this research therefore, was to evaluate the performance of the automated AiDx_NTDx Assist (an artificial-intelligence-based digital pathology) for the detection of microfilaria – *W.bancrofti* in prepared blood samples (glass slides) using standard microscopy as a reference.

The research objective is stated:

- To carry out detection and quantification of microfilaria using the automated AiDx_NTDx microscope in samples obtained from the field and evaluate the performance of the device with respect to the WHO performance metrics (Sensitivity, Specificity, PPV and NPV) using manual microscopy as the reference standard.

- To technically evaluate the automated AiDx NTDx assist with respect to the published WHO Target Product Profile for diagnosis of Lymphatic Filariasis.

AIM OF THIS STUDY

The aim of this research was to evaluate the performance of the automated AiDx_NTDx (an artificial-intelligence-based digital pathology) for the detection of microfilaria – *Wuchereria bancrofti* in prepared blood samples (glass slides) using standard microscopy as a reference.

RESEARCH OBJECTIVE

- To carry out detection and quantification of microfilaria using the automated AiDx_NTDx microscope in samples obtained from the field and evaluate the performance of the device with respect to the WHO performance metrics (Sensitivity, Specificity, PPV and NPV) using manual microscopy as the reference standard.
- To technically evaluate the automated AiDx NTDx assist with respect to the published WHO Target Product Profile for diagnosis of Lymphatic Filariasis.

Ethical approval

SHA 52/VOI.XII/99

1.1 ETHICAL CONSIDERATION AND CONSENT

Ethical approval (SHA 52/VOI.XII/99) was given by the Ogun State Hospital Management Board, State Hospital Sokenu, Abeokuta Ogun State Nigeria. Pre-advocacy meetings were held with all levels of leadership in the four selected Local Government Areas to inform, sensitize, and create the community awareness about the study. Their consent and approval were given and documented. Also, written informed consent was obtained from all study participants above the age of 18 years. Parental consent for children below the age of 18 years was also obtained.

METHODOLOGY

2.1 STUDY SITE

Study sites in the 3 Senatorial District of Ogun state were selected based on the prevalence of LF in the communities. Selected LGA include: Adodo Ota with a prevalence of 10%, Yewa North - 4.1%, Ijebu Ode -8% and Abeokuta North – 4%. Selection of study communities was based on a previous LF prevalence mapping by the Federal Ministry of Health. The mapping survey was conducted in the year 2013 following WHO recommendations accordingly [13].

Participants who were 18 years and above that gave consent were included into the study while those who did not give consent were excluded. Considering the general level of insecurity in the study areas, participants were transported from their communities to a pre-selected safe primary health care center for sample collection where they stayed overnight. Participants had opportunity of withdrawing from any part of the study if they felt uncomfortable.

2.2 SAMPLE SIZE AND SAMPLING TECHNIQUE

The sampled LGAs were selected based on their high prevalence with LF according to the FMOH's mapping result (2013,). The Cochran formu$(N_O=\frac{Z^2pq}{e^2})$ was used to determine the sample size for each of the study locations to ensure that sufficient positive cases were sampled. Where 'e' is the desired level of precision at 95% confidence (i.e., the margin of error), 'p' is the baseline prevalence (estimated proportion of the population which has the attribute in question) and q is 1 – p. While the Z values at 95 % confidence level gives us 1.96 (Z-table). Given the baseline prevalence we computed a sample size distribution of 500 samples from Adodo Ota (LGA with highest LF prevalence) and 250 samples from each of the other LGA's.

Laboratory scientists and recorders from the NTD department of the Ogun State Ministry of Health were trained before the commencement of sample collection. Finger prick blood samples (60 each in non-heparinized capillary tube) were collected from each participant. A total of 1,250 samples was collected for analysis. Blood samples were collected in the night between the hours of 22:00 to 02:00. The finger-pricked capillary blood collected from participants were smeared on a clean glass slide and allowed to air dry after which they were packed in labeled slide boxes and taken to the laboratory in Abeokuta for preparation. The dried blood samples were then dehaemoglobinised using clean water and air dried thereafter after which they were fixed with absolute methanol and stained in Giemsa mixed with phosphate buffer 7.2 for 30 minutes. After 30 minutes, the samples were air dried and stored in slide boxes for microscopy analysis. The prepared blood smears were then transported to Abuja and analyzed by the first expert LF scientist. Results recorded were privately shared with the principal Investigator who did not disclose the outcome of the first analysis with both the AiDx operator and the second expert LF scientist. The AiDx NTDx Assist was then used to analyze the samples and recorded results were documented and independently shared with the PI. Finally, the slides were transported to the University of Jos, where the second expert microscopy reader analyzed the samples. Expert LF scientist with capacity to produce reliable microscopy results are not commonly available hence the need to move samples from one place to another for expert analysis.

2.3 TECHNICAL SPECIFICATIONS OF AIDX DEVICE

The multi-diagnostic AiDx_NTDx Assist device is a low-cost and compact automated microscope with an inbuilt slide-scanner and an on-board computer for system control. The hardware system comprises of custom designed optics, automated slide scanner with 3 degrees of freedom along the (x, y and z) axis respectively, electronic and computing modules. The spatial resolution and magnification of the imaging platform is sufficient to clearly resolve target parasites of interest such as microfilariae in blood sample, *S. haematobium* eggs in urine and *S.mansoni* & soil-transmitted helminths (STHs) eggs in fecal matters. Digitally registered images are stored up and can be re-accessed or reused when needed. Registered images are screened using an integrated proprietary Artificial Intelligence (AI) model which identifies the microfilaria in blood and *S.haematobium* urine samples respectively. Algorithm to detect *S.mansoni* and STHs is currently being developed.

Mapping result of Ogun state showing the endemicity/non-endemicity level in the 20 Local Government Areas. Mapping was done by the NTD program of the Nigerian Federal Ministry of Health.

S/No	State	LGA	Total Population	Mapping Information	Endemicity Classification	Level of Endemicity	Year of Engagement
1	Ogun	Abeokuta North	277,534	Yes	endemic	2.1	2014
2	Ogun	Abeokuta South	345,011	Yes	endemic	4.0	2014
3	Ogun	Ado-Odo/Ota	725,876	Yes	endemic	10.0	2014
4	Ogun	Egbado North (Yewa North)	250,649	Yes	endemic	4.1	2014
5	Ogun	Egbado South (Yewa South)	232,762	Yes	endemic	3.2	2014
6	Ogun	Ewekoro	76,033	Yes	endemic	4.0	2014
7	Ogun	Ifo	723,494	Yes	non-endemic	0.0	
8	Ogun	Ijebu East	151,906	Yes	endemic	2.1	2014
9	Ogun	Ijebu North	391,960	Yes	non-endemic	0.0	
10	Ogun	Ijebu North East	93,234	Yes	non-endemic	0.0	
11	Ogun	Ijebu Ode	212,335	Yes	endemic	8.0	2014
12	Ogun	Ikenne	163,678	Yes	non-endemic	0.0	
13	Ogun	Imeko Afon	113,337	Yes	endemic	2.3	2014
14	Ogun	Ipokia	207,364	Yes	endemic	2.1	2014
15	Ogun	Obafemi-Owode	315,474	Yes	endemic	2.0	2014
16	Ogun	Odeda	150,877	Yes	non-endemic	0.0	
17	Ogun	Odogbolu	175,240	Yes	endemic	1.0	2014
18	Ogun	Ogun Waterside	100,542	Yes	non-endemic	0.0	2014
19	Ogun	Remo north	82,588	Yes	endemic	1.0	2014
20	Ogun	Shagamu	349,331	Yes	endemic	1.0	2014

RESULTS

Performance Evaluation Results

During this evaluation, a total of 1250 participants were examined for microfilariae (Mf). The demographics of the participants is shown in (Table 2). Of the 1250 participants, 502 are male and 748 are female. The participants are > 5 years of age. While 637 are aged 40 and above, 613 are less than 40 years of age. None of the examined blood smears had detectable W. bancrofti microfilariae.

DEMOGRAPHICS & DATA DISTRIBUTION OF SAMPLED POPULATION IN OGUN STATE.

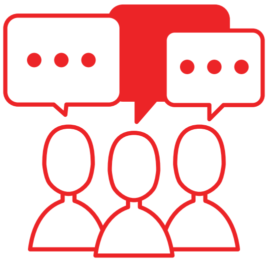
LGA	Sex	Number Tested	Age Group (Years)					No. of +ve (%)
			<11	11-20	21-30	31-40	>40	
Abeokuta South	Male	106	0	7	15	13	71	0 (0.0)
	Female	144	0	11	15	27	91	0 (0.0)
Ado Odo Ota	Male	169	5	50	21	23	70	0 (0.0)
	Female	331	7	85	73	69	97	0 (0.0)
Ijebu Ode	Male	124	0	2	18	19	85	0 (0.0)
	Female	126	1	10	14	22	79	0 (0.0)
Yewa North	Male	103	0	5	14	17	67	0 (0.0)
	Female	147	0	11	21	38	77	0 (0.0)
Total		1250	13	181	191	228	637	0 (0.0)

Since no positive samples was detected by the reference microscopy and the AiDx NTDx Assist, it was impossible to estimate the sensitivity of the device. However, based on the negative results obtained, the AiDx Assist showed a specificity of 100%, an accuracy of 100% and a Negative Predictive Value of 100%. Outcome of AiDx assist sample analysis with respect to the reference standard is presented in table 3.

ANALYSIS OF THE EXPERT MICROSCOPY RESULTS COMPARED TO THE AIDX ASSIST RESULT.

S/No	Detail of Analysis	Expert Microscopy Result	AiDx Assist Result
1	Number of Positive cases detected	0	0
2	Number of Negative cases detected	1250	1250
3	Negative Predictive Value	N/A	100%
4	Specificity	N/A	100%
5	Accuracy	N/A	100%

Discussion



3.1 DISCUSSION

Image based microscopy analysis for the detection of microfilariae in prepared blood smears carried out in November 2022 in four LGA's of Ogun state using expert LF microscopy and AiDx NTDx Assist demonstrates that none of the 1250 participants had detectable microfilaria in the analyzed blood sample.

This significantly contradicts the mapping results of the NTD program from the Federal Ministry of Health in the study area. The mapping survey conducted in 2013 (approximately 9 years prior) showed a prevalence level that varies between (4% and 10%) in the selected LGA's. Although the mapping data available is quite old, this possibly shows a need to update the current national mapping of LF in the country. The result obtained in this study is in concordance with a study conducted by [14]. Out of 986 subjects examined in that study, none of the subject showed a detectable level of Circulating Filaria Antigens.

From the experience in the study, we can possibly infer that vector control interventions implemented between previous mapping and current study could account for the zero results obtained in our study. Common vector control interventions in Ogun state include the distribution and active use of insecticide treated nets. Furthermore, variance between the result obtained in this study and the mapping results could also be attributed to LF treatment and intervention provided in the state by the NTD department of the Federal Ministry of Health in 2018. Whatever the case, the result reported in this study demonstrates that a gap can be complemented using reliable diagnostic devices like the AiDx NTDx Assist devices. Re-assessing the prevalence of W. bancrofti infection after a couple of years is therefore recommended.

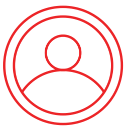
Matching AiDx NTDx assist Device with the WHO Target Product Profile

4.0 PRODUCT USE SUMMARY

The AiDx NTDx Assist microscope is suitable for use both in the laboratory and outside the standard laboratory environment. The device can be used during survey in remote areas or for awareness campaign purposes.

4.1 LOWEST INFRASTRUCTURE LEVEL

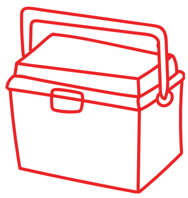
The AiDx NTDx Assist microscope is a ready to use system with an integrated battery back-up system. A version with an integrated processing system and monitor has been temporarily discontinued due to the shortage in the semi-conductor industry. A newly developed laptop-based system provides more processing power, and better user experience. System can therefore be used at remote areas without laboratory infrastructure without much dependency on stable electricity. Although internet connectivity can be achieved through hotspot connections, results are generated offline and can be stored without any reliance on cloud computing.



4.2 LOWEST LEVEL OF USER

The AiDx NTDx Assist Microscope require experienced or properly trained operators for collection and preparation of blood samples as demonstrated in this study. From the experience in this study, one day of training was sufficient to prepare a laboratory technician for blood sample collection and preparation on the field. Tests can be performed by low-skilled health personnel and field workers. The educational, and technical requirement for the device use and result interpretation is grossly minimal. The device can be used by anyone with basic general knowledge (with the ability to operate a smartphone).

4.3 PORTABILITY



In the case of point-of-sampling tests in remote locations, the device is sufficiently portable for easy transportation. The device has survived several robustness, shock, and vibration tests. Also, device is robust and safe for operation under temperatures of up to 40 degrees. The system is safely protected from humidity and properly covered to prevent dust on the internal optical surfaces. A standard system weighs 15kg and can fit into a simple carry-on flight luggage.System has been easily transported to remote areas in Nigeria and Gabon in vehicles and bicycles. and has demonstrated sufficient robustness to survive the harsh weather and road conditions.

.

4.4 MAINTENANCE AND CALIBRATION

Although regular maintenance and hardware recalibration is not critical in the first 2 years, AiDx Medical BV has trained local partners in Nigeria to provide basic routine technical support and training at regular intervals. Onboard software calibrations are integrated to ensure stability and consistency in the auto-focus algorithm. This support and maintenance model can be reproduced in other countries providing job opportunities for the locals as well.

4.5 DETECTION OF TARGET ANALYTE AND EASE OF RESULTS INTERPRETATION

The biomarker used by the AiDx Assist NTDx is the Wuchereria bancrofti (W.b) microfilaria. The system detects W.b from the registered images and provides annotated output of the suspected parasite for the operator for further diagnostic conclusions. The microfilaria can be viewed using both 4x and 10x magnification. Approximately 330 and 1200 fields of view (FOV) are registered accordingly. The interpretation of registered images is performed by an integrated AI software. The AI outputs detected features which is further validated by a trained operator. The AI device also provide an approximate value of the parasitemia in each examined sample.

4.6 DIAGNOSTIC/CLINICAL SENSITIVITY

Unfortunately, the sensitivity of the AiDx NTDx device for the detection of W.b could not be estimated from the data obtained from this study. However, the device demonstrated a specificity of 100%.

4.7 TIME TO RESULT

Set-up of the system by trained personnel at the lowest infrastructure level is less than 15 minutes. Scanning of single blood smear can be actualized in less than 10 minutes with the 4x magnification and in 20 minutes with the 10x magnification AiDx device. Total time of sample analysis includes, sample scanning, image registration and data analysis with embedded artificial intelligence. The system output provides additional information on the estimated infection load. Result obtained are stored on the system and are easily transferable to a portable hard disk or any other simple electronic storage device.

4.8 DATA STORAGE AND MAPPING

For geospatial tagging of sample, which is useful for disease mapping, the current AiDx device has a software interface that requests for the location of sample collection. The input to this request is automatically tagged on all scanned samples collected in the area.

4.9 CAPITAL COST

The current AiDx NTDx Multidiagnostic device that can optically detect schistosomiasis, STH, LF and Loaloa is currently available at a market price of US $5,250 for a complete system (laptop inclusive).

Limitation of Study

One of the limitations of this study is that there was no positive case to evaluate the sensitivity of the AiDx NTDx Assist. Also, the use of an antigen based circulating filarial test could have possibly provided basis for comparisons that could provide more insight into the validation of the result. Conclusively, the security challenges experienced in the country during the sample collection period for this study restricted the movement of the sample collection team to primary health facilities. It is possible that interior locations with potential active LF cases were inaccessible.

References

1. WHO, author. Lymphatic filariasis: The disease and its control. Fifth report of the WHO expert committee on filariasis. Geneva: 2002. [PubMed]
2. Nwoke BEB, Mberu BU, Oha O, Dozie INS, Ukaga CN. Clinical and parasitological studies. Geneva: WHO; 2000. Lymphatic filariasis and Onchocerciasis in rain forest of southern Nigeria I. WHO/TDR/ID:981087.
3. Ottesen EA. The global programme to eliminate lymphatic filariasis. Trop Med Int Health. 2000;5:591–594. [PubMed] [Google Scholar]
4. Ottesen EA, Duke BOL, Karam M, Behbehani K. Strategies and tools for the control/elimination of lymphatic filariasis. Bull World Health Organ. 1997;75:491–503. [PMC free article] [PubMed] [Google Scholar]
5. Molyneux DH, Hotez PJ, Fenwick A. “Rapid-impact interventions”: how a policy of integrated control for Africa's neglected tropical disease could benefit the poor. PloS Med. 2005;2:e336. [PMC free article] [PubMed] [Google Scholar]
6. King CL, Suamani J, Sanuku N, Cheng YC, Satofan S, Mancuso B, et al. A Trial of a Triple-Drug Treatment for Lymphatic Filariasis. N Engl J Med. 2018; 379(19):1801–10. https://doi.org/10.1056/NEJMoa1706854 PMID: 30403937
7. Hamlin KL, Moss DM, Priest JW, Roberts J, Kubofcik J, Gass K, et al. Longitudinal monitoring of the development of antifilarial antibodies and acquisition of Wuchereria bancrofti in a highly endemic area of Haiti. PLoS Negl Trop Dis. 2012; 6(12):e1941. https://doi.org/10.1371/journal.pntd.0001941 PMID:23236534
8. Dubray CL, Sircar AD, Beau de Rochars VM, Bogus J, Direny AN, Ernest JR, et al. Safety and efficacy of co-administered diethylcarbamazine, albendazole and ivermectin during mass drug administration for lymphatic filariasis in Haiti: Results from a two-armed, open-label, cluster-randomized, community study. PLoS Negl Trop Dis. 2020; 14(6):e0008298. https://doi.org/10.1371/journal.pntd.0008298 PMID: 32511226
9. Lammie PJ, Weil G, Noordin R, Kaliraj P, Steel C, Goodman D, et al. Recombinant antigen-based anti-body assays for the diagnosis and surveillance of lymphatic filariasis—a multicenter trial. Filaria J.2004; 3(1):9. https://doi.org/10.1186/1475-2883-3-9 PMID: 15347425
10. Noordin R, Muhi J, Md Idris Z, Arifin N, Kiyu A. Duration of detection of anti-BmR1 IgG4 antibodies after mass-drug administration (MDA) in Sarawak, Malaysia. Trop Biomed. 2012; 29(1):191–6. PMID:22543621
11. Rahmah N, Lim BH, Khairul Anuar A, Shenoy RK, Kumaraswami V, Lokman Hakim S, et al. A recombinant antigen-based IgG4 ELISA for the specific and sensitive detection of Brugia malayi infection.Trans R Soc Trop Med Hyg. 2001; 95(3):280 https://doi.org/10.1016/s0035-9203(01)90234-2 PMID:11490997
12. Won KY, Gass K, Biamonte M, Dagne DA, Ducker C, Hanna C, et al. (2021) Diagnostics tosupport elimination of lymphatic filariasis—Development of two target product profiles. PLoSNegl Trop Dis 15(11): e0009968. https://doi.org/10.1371/journal.pntd.0009968
13. WHO (2000) Operational guidelines for rapid mapping of bancroftian filariasis in Africa. WHO/CDS/CPE/CEE/2000.9. Geneva, Switzerland: World Health Organization.

14. Pam, D. D., de Souza, D. K., D'Souza, S., Opoku, M., Sanda, S., Nazaradden, I., ... & Koudou, B. G. (2017). Is mass drug administration against lymphatic filariasis required in urban settings? The experience in Kano, Nigeria. PLoS neglected tropical diseases, 11(10), e0006004.
World Health Organization, 2021. Diagnostic test for surveillance of lymphatic filariasis: target product profile.
15. World Health Organization, 2021. Diagnostic test for lymphatic filariasis to support decisions for stopping triple-therapy mass drug administration: target product profile.
Ward, P., Broadfield, L.A., Dahlberg, P., Leta, G., Mekonnen, Z., Nabette, B., Kabatereine, N.,
16. Brooks, A., Orrling, K.M., Gomes, M. and Van Hoecke, S., The development of an artificial intelligence-based digital pathology for neglected tropical diseases: a platform specific analysis of the World Health Organization diagnostic target product profile for soil-transmitted helminthiasis.
17.. Frontiers in Tropical Diseases, p.118.

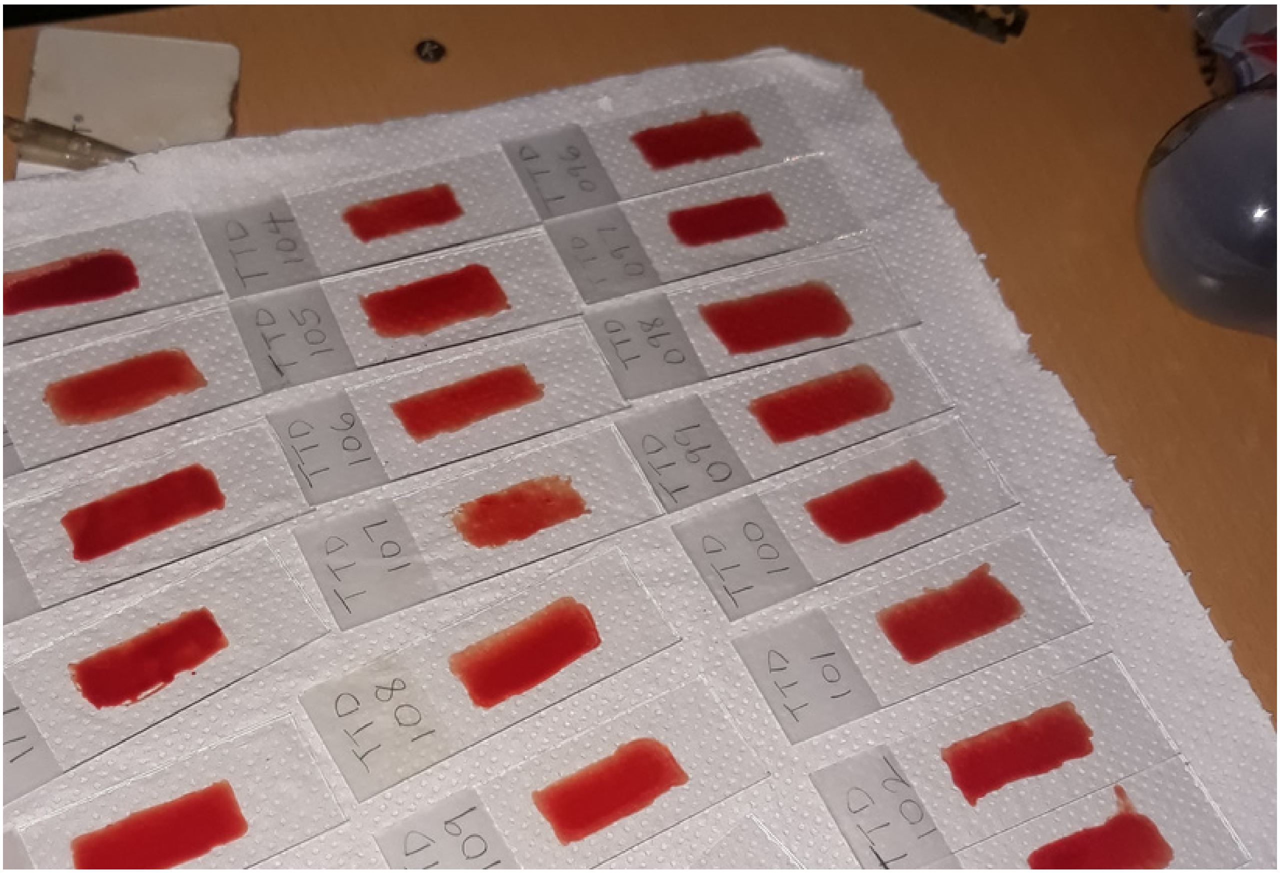
APPENDIX 1:

PICTURES OF ADVOCACY & SAMPLE COLLECTION FOR LF IN OGUN STATE.

Pres-study advocacy at by NTD team , Ogun state Ministry of Health Nigeria at the palace of the Olu of Igbogila - Yewa North

Training session ogranised for members of the team in Ogun State.

Sample collection Adodo Ota



Sample collection.

Sample collection - Joseph Kumbur and Team Ogun NTD - Abeokuta South

Ogun NTD team collecing samples at night on the field at Yewa North

Sample collection - Ijebu Ode